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Epithelial stem cells of the lung: privileged few or opportunities for many?

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Most reviews of adult stem cells focus on the relatively undifferentiated cells dedicated to the renewal of rapidly proliferating tissues, such as the skin, gut and blood. By contrast, there is mounting evidence that organs and tissues such as the liver and pancreatic islets, which turn over more slowly, use alternative strategies, including the self-renewal of differentiated cells. The response of these organs to injury may also reveal the potential of differentiated cells to act as stem cells. The lung shows both slow turnover and rapid repair. New experimental approaches, including those based on studies of embryonic development, are needed to identify putative lung stem cells and strategies of lung homeostasis and repair.

Introduction

Throughout adult life, multicellular organisms must generate new cells to maintain the structure and function of their tissues. In young animals, tissue damage can usually be repaired quickly, but this natural capacity may fail after repeated challenges and with age. Diseases such as cancer may usurp and exploit the mechanisms by which the body normally rebuilds itself. These considerations drive us to understand the mechanisms by which adult organs normally achieve tissue homeostasis and repair. The emerging picture is that different organs use different strategies to renew themselves, and that more diversity and flexibility underpin these renewal processes than previously imagined.

Some organs, such as hair follicles, blood and gut, which constantly renew themselves throughout life, contain adult stem cells that are morphologically unspecialized, have a relatively low rate of division and are topologically restricted to localized regions known as 'niches' that tightly regulate their behavior (Fuchs et al., 2004; Lanza, 2006). These 'dedicated' stem cells (see Box 1) undergo long-term self-renewal. They also produce a population of transit amplifying (TA) daughter cells (see Box 1) that have a high rate of proliferation, can self-renew over the short term and give rise to precursors of all or many of the differentiated cell types of the organ. These concepts are now well established (Fig. 1). However, recent research has emphasized that the classical hierarchy of the tissue-specific stem cell, TA cells and differentiated cells is not always rigid and irreversible (Raff, 2003). For example, the commitment of cells to a specific fate may occur gradually, so that if stem cells are ablated, some early TA cells may enter the empty niche and function as stem cells (Kai and Spradling, 2004; Potten, 2004). TA cells may also be able to change their fate to give rise to cells of another tissue type when exposed to appropriate signals. One striking example occurs when cells from the central cornea of the adult rodent eye, well away from any stem cells, are grafted onto dermis from embryonic skin. Over several days they proliferate, express different genes and generate hair follicles and sebaceous glands, presumably with associated stem cells (Pearton et al., 2005). This process is known as 'transdifferentiation' – a term that needs careful use according to its context (see Box 2).

In contrast to rapidly renewing organs such as the skin and gut, some organs apparently maintain themselves without the aid of an undifferentiated stem cell population. Evidence for this concept comes from recent experiments in which insulin-producing β-cells of the adult mouse pancreas were labeled with a heritable genetic marker and followed during normal turnover and regeneration after partial pancreatectomy (Dor et al., 2004). Likewise, in the liver, turnover and regeneration after hepatectomy involves the division of differentiated hepatocytes. However, if hepatocyte proliferation is inhibited, interlobular bile duct cells can replenish the hepatocyte population (Alison et al., 2004). Such observations have engendered the concept of 'facultative' stem cells – normally quiescent differentiated cells that can act as stem cells after injury, perhaps by recapitulating processes that are active during development (see Box 1).

The adult lung is a vital and complex organ that normally turns over very slowly. The epithelial cells that line the airways are constantly exposed to potential toxic agents and pathogens in the environment, and they must therefore be able to respond quickly and

Box 1. A glossary

Dedicated stem cell A relatively undifferentiated cell present in the adult organ, usually in localized niches. It normally divides infrequently; is capable of both long-term ('lifetime') self-renewal and of giving rise to daughter cells that differentiate into one or more specialized cell type; and it functions in both tissue homeostasis and repair.

Facultative stem cell Differentiated cell that is normally quiescent but responds to injury by dividing and self-renewing, and giving rise to progeny that differentiate into one or more cell types.

Metaplasia Strictly, the process by which a stem or progenitor cell of one tissue switches to become a progenitor of cells of another tissue type.

Post-mitotic differentiated cell A cell that can no longer divide and must be replenished during normal turnover or injury.

Progenitor cell Either a cell in the developing organ, usually multipotent, that is the source of an initial population of adult cells before turnover begins, or, more loosely, a cell that gives rise to another cell. Cell lineage relationships during development may not necessarily reflect those that occur during repair.

Self-renewing differentiated cell Differentiated cell that divides and self-renews over the long term. Functions in both normal tissue homeostasis and in response to injury.

Transdifferentiation See Box 2.

Transit amplifying (TA) cell An intermediate between a dedicated stem cell and its final differentiated progeny. Can proliferate, self-renew over the short term and give rise to one or more differentiated cell type.

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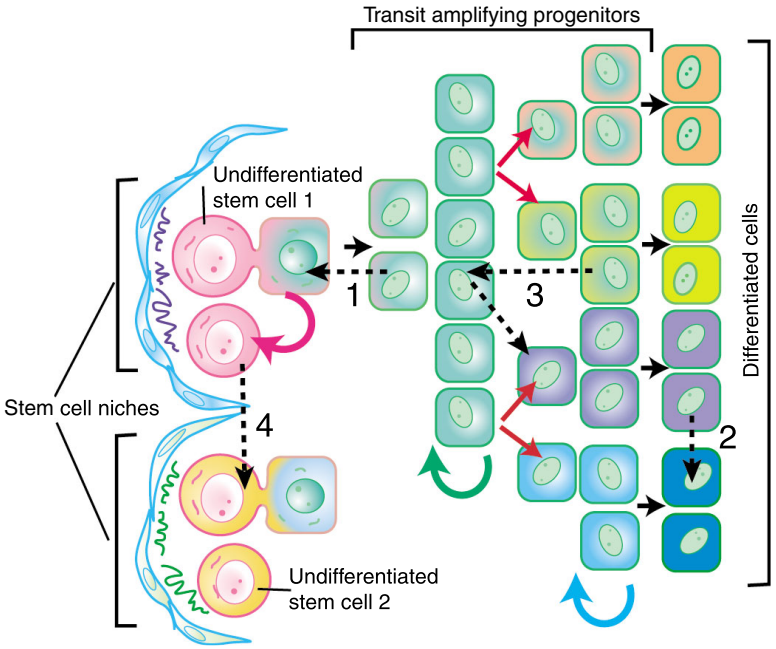

Fig. 1. Classical stem cell hierarchy. Model of the 'classical' hierarchy of undifferentiated epithelial stem cell, transit amplifying (TA) progenitor cells and mature postmitotic differentiated cells. Cell fate choices are indicated by red arrows. In this model, the stem cell in its 'niche' and different TA cell subclasses can self-renew (curved arrows). Stem cells self-renew infrequently and TA cells more rapidly. Early TA cells may be able to replace stem cells if the niche is depleted (dashed arrow 1). The niche probably consists of several cell types and associated molecules, including blood vessels and nerves. 'Transdifferentiation' of one well-defined differentiated cell type into another could occur directly, without cell division (dashed arrow 2) or might also involve reversion or de-differentiation between distinct TA progenitor populations (dashed arrows 3). Rarely, stem cells switch from one tissue-specific lineage to another (dashed arrow 4) in a process called metaplasia or transdetermination (see Box 1). Adapted, with permission, from Watt and Hogan (Watt and Hogan, 2000).

effectively to both cellular damage and to the local production of immune cytokines. Over the years, several experimental protocols have been developed in mice that mimic the injuries and rapid repair processes elicited in the lung by environmental challenges. The picture that is emerging from these models is that different regions of the respiratory system – the trachea and large airways, and the distal bronchioles and alveoli – use different kinds of stem cells and strategies for maintenance and repair. Moreover, there is evidence that differentiated epithelial cell types are able to proliferate and transdifferentiate in response to some conditions. However, the precise mechanisms involved in any of these processes are still very unclear.

This review summarizes some recent findings, and outlines the challenges and opportunities for future research into lung turnover and repair. These findings are likely to be relevant to other organ systems that do not exactly fit the well-described examples of skin/bone marrow/gut and islets/liver. In particular, we suggest how concepts and tools familiar to developmental biologists may help elucidate some of the outstanding problems. Examples include the use of new technologies for lineage analysis in mice (Metzger and Chambon, 2001), and tissue recombination experiments to test the developmental potential of isolated cells (Pearton et al., 2005; Xin et al., 2003; Xin et al., 2005). Furthermore, work on the reciprocal interactions between mesenchymal, endothelial and epithelial cells during development will inform studies on the interplay between niche cells and stem cells in adult tissues (Lammert et al., 2003). Finally, studies into the role of cell shape changes, asymmetric cell division and cell-matrix interactions in regulating gene expression in the embryo may help us to elucidate the changes that are taking place in adult tissues when they are undergoing repair and regeneration (Keller, 2005; Li et al., 2003; Nguyen et al., 2005).

Structure of the normal adult lung epithelium

The lung has a complex three-dimensional structure that features major differences along its proximodistal axis in terms of the composition of the endoderm-derived epithelium (Fig. 2). The trachea and primary lung buds arise by different morphogenetic processes from contiguous regions of the embryonic foregut (Cardoso and Lu, 2006). In the adult mouse trachea and primary bronchi (cartilaginous airways), the luminal epithelium contains two main columnar cell types: ciliated cells and Clara-like cells (Fig. 2). The latter produce secretoglobins, the most abundant of which is Scgb1a1 (also known as CCSP, CC10 or CCA). A small number of innervated neuroendocrine (NE) cells are also present. In the adult mouse, mucous-producing cells are restricted to a few submucosal glands in the upper airway. The epithelium of the submucosal glands is continuous with the luminal epithelium and contains ciliated and basal cells. A distinguishing feature of the cartilaginous airways is that they contain a discontinuous population of relatively unspecialized basal cells that express p63 (Trp63 – Mouse Genome Informatics) and specific keratins (K14 and K5). These cells do not appear in the trachea until around birth and after the differentiation of ciliated and secretory cells. Tracheas of $p63^{-/-}$ embryos lack basal cells but do have a columnar epithelium that contains predominantly ciliated cells (Daniely et al., 2004).

In the more distal airways (small bronchi and bronchioles) (Fig. 2), the epithelium is columnar. Clara cells predominate over ciliated cells and there are more NE cells than in the trachea. Importantly, however, there are no basal cells, so that they cannot be involved in local turnover and repair (Pack et al., 1981).

The most distal region of the lung is organized into a complex system of alveoli (Fig. 2). There are two types of epithelial cell here: type I cells, which provide the thin-walled gas exchange surface; and cuboidal type II cells. The latter contain numerous secretory vesicles (lamellar bodies) filled with surfactant material, including surfactant-associated protein C or Sftpc. The transitional region between the terminal bronchioles and the alveoli is known as the bronchioalveolar duct junction (BADJ).

Currently, the identification of these different epithelial cell types relies largely on the expression of secreted proteins, which is not ideal for sorting cells by flow cytometry. Progress is being made in

Box 2. Transdifferentiation

This refers to the transformation of one well-defined type of fully differentiated cell into another well-defined type. In this review, we refer to 'direct transdifferentiation' as the transformation from one phenotype to another without an obligatory round of cell proliferation. 'Transdifferentiation with proliferation' requires at least one intervening round of cell proliferation. The direct mechanism is more likely to involve the transient existence of a cell that co-expresses differentiation markers of both old and new phenotypes. By contrast, the mechanism that involves proliferation is more likely to involve the transient existence of a 'de-differentiated' cell, which has a pattern of gene expression that is different from either the initial or final cell type. Some stem cell researchers have applied the term 'transdifferentiation' to 'the ability of a particular cell of one tissue…including stem or progenitor cells, to differentiate into a cell type characteristic of another tissue' (see www.isscr.org/glossary/index.htm). This much looser definition really describes a phenomenon that is known as 'transdetermination' when it occurs in embryos and as 'metaplasia' when it occurs in adults.

finding better markers for distinguishing and sorting different lung epithelial cell types (e.g. Kim et al., 2005; Reynolds et al., 2002). However, there may be important subpopulations of cells that are missed at present. We are a long way from having a molecular signature for each of the cell types of the adult lung and there is a pressing need for a comprehensive public database of cell type gene expression.

Lung turnover is normally slow

Estimates for the turnover time of the mouse airway epithelium vary widely. This may reflect differences in the health and pathogen status, strain and age of individuals (Wells, 1970). Lung size in rodents keeps constant pace with body size, so that mouse lungs continue to grow throughout life, although the rate slows with age (Thurlbeck, 1975). However, the consensus is that the turnover time of the tracheal-bronchial epithelium of adult rodents is more than 100 days (4 months) (Blenkinsopp, 1967). By comparison, the gut, which is also endodermally derived, has an estimated turnover time of 4 days.

The clearest studies of steady-state maintenance of the lung have involved pulse/chase experiments with tritiated thymidine ([^{3}H]-TdR). In the proximal airways (trachea/bronchi), this approach has identified basal and secretory (Clara-like) cells as the dividing cells and the ciliated cells as their descendents (Breuer et al., 1990; Donnelly et al., 1982; Kauffman, 1980). However, this method cannot be used to determine conclusively whether it is the basal or Clara cells that give rise to the ciliated cells and whether the relationship is the same in different regions of the lung. ([^{3}H]-TdR incorporation at steady state in airways lacking basal cells has not been closely studied.) The best way to define lineage relationships is to use Cre/lox genetic labeling to follow the descendants of specific cells. For example, the inducible expression of Cre recombinase could be used to activate a heritable reporter gene specifically in Scgb1a1-expressing (Clara and Clara-like) cells and to ask which cell types subsequently acquire the label. In addition, one can ask whether there is a substantial dilution of the label in the population over time, as expected if Clara-like cells are continually replaced from a pool of unlabeled stem cells (Dor et al., 2004).

'Mucous metaplasia' in the adult lung

Genetic lineage tracing would also help to clarify the current confusion over the mechanisms that underlie the phenomenon loosely called 'mucous metaplasia' that is seen in adult mice challenged with aerosolized allergens, and probably involves the local production of inflammatory cytokines, such as interleukin 13 (Williams et al., 2006). In the normal adult mouse lung, there are few mucous-secreting cells outside the submucosal glands, although some are seen in the early postnatal lung (Rawlins and Hogan, 2005). However, a few days after antigen exposure, large numbers can be found in the trachea and large airways. One idea is that these are derived by the 'direct transdifferentiation' (see Box 2) of ciliated cells or Clara cells, as cells have been observed by electron microscopy that contain both cilia and large numbers of mucous-containing vesicles (Evans et al., 2004; Hayashi et al., 2004; Reader et al., 2003; Tyner et al., 2006). Some of the other hypotheses are that the formation of mucous cells involves the differentiation of progenitor cells, and/or the proliferation and de-differentiation of differentiated cells. Distinguishing between these mechanisms would improve our understanding of the origin of the large numbers of goblet cells that are found in humans with conditions such as chronic asthma.

Evidence for lung stem cells from ex vivo studies

In the hematopoetic system, it is possible to test the ability of cells to restore all the blood cell lineages by injecting them intravenously into an irradiated host. Likewise, dissociated hepatocytes can repopulate the damaged liver after injection into the portal vein, and clonal analysis can be achieved in this system using retrovirally labeled cells (Overturf et al., 1999). Recent studies have shown that a complete mouse mammary gland can be grown from a single adult epithelial cell implanted into a mammary fat pad (Shackleton et al., 2006). There are currently no such in vivo tests for the potency of lung cells. However, two ex vivo systems have been used to examine the regenerative potential of isolated lung epithelial cells: the rat tracheal xenograft model and cell culture. These systems are particularly useful because they can be applied to the study of human adult and fetal airway epithelial cells, including tracheal cells and nasal polyps, as discussed below (Dupuit et al., 2000).

Rat tracheal xenograft model

In this model, epithelial cells isolated from a donor airway epithelium are dissociated and seeded onto the surface of a host rat trachea that has been denuded of endogenous epithelial cells by freeze-thawing. The trachea is then grafted subcutaneously into an immunodeficient mouse. Several weeks later, a well-differentiated, normal airway epithelium with a few submucosal glands is restored (Fig. 3); whether this organization can be maintained over the long term is not known. Two kinds of studies have been carried out with this model. First, retroviral labeling has been used to follow retrospectively the proliferative and differentiation potential of single epithelial cells within the seeding population. When adult human bronchial epithelial cells were used, the largest and most frequently labeled clones contained basal, ciliated and goblet cells. However, clones consisting of subsets of these cell types also formed (Engelhardt et al., 1995). These results illustrate that there are both multipotent cells and lineage-restricted progenitor cells in the human airway epithelium.

The second experimental approach has been to sort the donor cells into basal and non-basal populations and then to follow their ability to reconstitute the surface epithelium. The results from such studies

have so far been very variable. Some suggest that both populations can restore the tracheal epithelium equally well (Avril-Delplanque et al., 2005; Liu et al., 1994). However, others have found that only columnar cells (Johnson and Hubbs, 1990) or only basal cells (Ford and Terzaghi-Howe, 1992) can restore all of the epithelial cell types. These discrepancies may be due to differences in sorting methods, donor species or the length of time allowed for epithelial repopulation. In spite of these differences, and the urgent need for better methods of sorting tracheal cell populations, these results suggest that both the columnar and basal cells can restore the tracheal epithelium in the xenograft model.

These xenograft studies are limited by the fact that the host tracheal mesoderm is dead and may not provide an ideal environment for the survival and differentiation of all donor lung cells. A potential alternative, which has been very informative in other organ systems, would be to place dissociated adult lung epithelial cells together with embryonic lung mesenchyme and then graft the recombinants under the kidney capsule of immunocompromised mice. In this environment, the grafts are readily vascularized. Indeed, recent studies in which intact embryonic lungs have been grown in this way have demonstrated that the close association between blood vessels and alveolar precursors, which is crucial for their normal development, occurs with high efficiency (Vu et al., 2003). The potential of this grafting technique is well illustrated by recent studies on stem cells and regeneration in the postnatal prostate epithelium, another endodermally derived tissue. By combining genetically marked and unmarked prostate epithelial cells with embryonic mesenchyme, it has been shown that an entire tubule could be derived from a single cell and that cells with regenerative capacity can be sorted based on surface markers (Xin et al., 2003; Xin et al., 2005). Like the trachea, the prostate contains p63-positive basal cells, although these are present before the appearance of differentiated secretory cells. Interestingly, if endodermal cells from a *p63*-null mouse mutant prostate are used in the graft, a proportion of the epithelial cells differentiate abnormally into mucous-producing cells (Kurita et al., 2004; Signoretti et al., 2005). This unexpected finding suggests that in the prostate, p63 is required in the basal cells to maintain the normal organization and fate of the columnar cells.

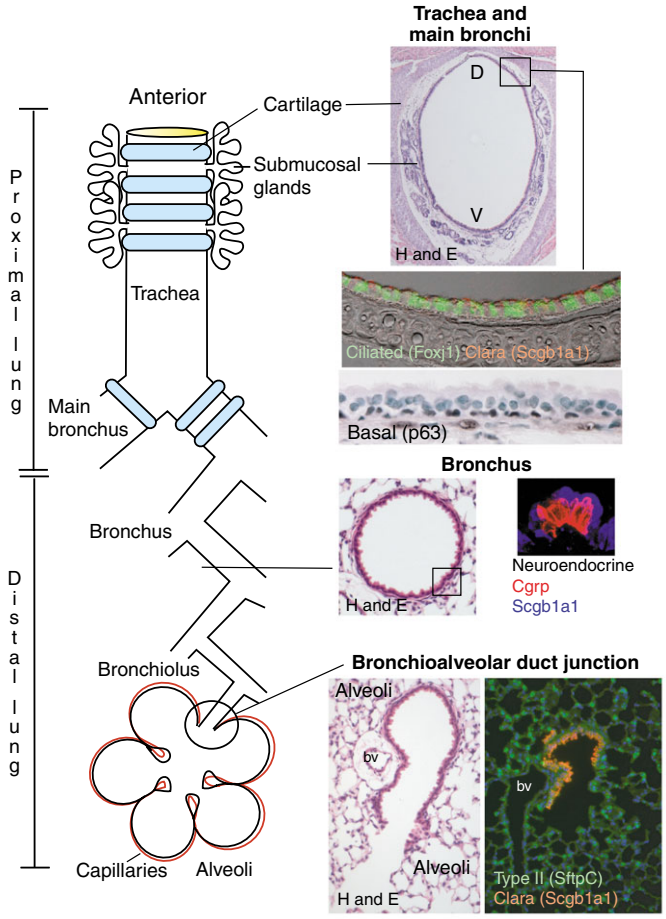

Fig. 2. A schematic of the main cell types along the proximodistal (rostrocaudal) axis of the mouse lung. Not all of the cartilage elements associated with the trachea and main bronchi are shown. Submucosal glands are present only in the upper part of the trachea in the mouse. The pseudostratified epithelium of the proximal airways contains mostly ciliated cells, which express the forkhead transcription factor Foxj1, Clara-like secretory cells [detected with antibody to Scgb1a1 (red)] and basal cells, detected with antibody to the transcription factor p63 (black). The bronchi also contain ciliated and Clara cells. They have many more neuroendocrine cells than the trachea, often in clusters at airway branch points, known as neuroendocrine bodies (NEBs), shown here by staining with antibody to calcitonin-gene related peptide (Cgrp, red). Each narrow bronchiolus opens into alveoli through a bronchioalveolar duct. This junction region is usually associated with a blood vessel (bv). The alveoli contain type II cells, which secrete large amounts of surfactant proteins [detected with antibody to surfactant protein C (Sftpc)]. The thin, flattened type I cells line the alveoli and are closely apposed by capillaries. Photographic images were all provided by E.R. from her own research material. The *FoxJ1GFP* transgenic mice used in one image were provided by Larry Ostwroski, University of North Carolina at Chapel Hill (NC, USA).

In vitro cell culture

In vitro culture offers many opportunities for studying epithelial stem cells. For example, the technique has long been used to assay the ability of adult epidermal keratinocytes to generate clonal colonies. Moreover, culture conditions have been developed that allow the massive expansion of human epidermal keratinocytes that support long-term survival and differentiation when grafted on to burn patients (Lanza, 2006). The development of in vitro clonogenic assays for lung epithelial cells is only in its infancy. The challenge has been to devise culture conditions in which adult airway epithelial cells, and in particular mouse cells, marked with a cell-autonomous genetic reporter, can reproducibly differentiate into secretory,

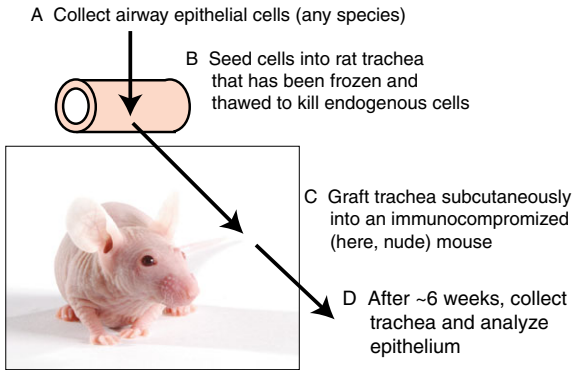

Fig. 3. A schematic of the rat tracheal xenograft model. Image of nude mouse courtesy Charles River Laboratories.

ciliated and basal cells. One system involves seeding dissociated airway epithelial cells on to membranes and culturing them at an air-liquid interface. By mixing genetically marked tracheal cells isolated from *Rosa26* mice (which ubiquitously express *lacZ*) with unmarked 'stuffer' cells, it has been possible to identify colonies derived from single cells. In these experiments, the largest colonies (derived from about 0.1% of the population) contain differentiated columnar epithelial cells, including ciliated cells, as well as basally orientated cells (Schoch et al., 2004). This assay system was also used to compare the proliferative capacity of basal and non-basal cells. Basal cells, sorted by flow cytometry on the basis of the expression of eGFP from a *keratin5* promoter, formed the larger colonies. Taken together, the results support the idea that the tracheal basal cells are stem cells in vivo. One of the main limitations of this assay is that it does not support the reproducible differentiation of all epithelial cell types, but future improvements may come from modifying culture conditions and using feeder layers.

In vitro culture has recently been used to test the differentiation potential of putative stem cells located at the BADJ of the mouse lung (Fig. 2). These cells have been termed bronchioalveolar stem cells or BASCs (Kim et al., 2005) (as discussed later in the review). Small numbers of cuboidal cells have been identified immunohistochemically in the BADJ that co-express both secretoglobin 1a1 (Scgb1a1, a secreted product of Clara cells) and surfactant protein C (Sftpc). Sftpc is a major secreted product of type II alveolar cells but is expressed, at low levels, by early embryonic lung epithelial cells (Khoor et al., 1994). The BASCs are also positive for the surface glycoprotein, CD34. This is commonly, but not exclusively, expressed on somatic stem cells, for example, on mouse hair follicle and hematopoietic stem cells. The Sftpc/Scgb1a1-positive cells were then cell sorted, based on being Sca1 positive, CD34 positive and being CD45 negative, Pecam negative. Single cells were cultured on a feeder layer of mouse embryonic fibroblasts, expanded and then cultured on a Matrigel substrate where they gave rise to cells that expressed markers for either Clara cells, type I cells or type II cells. Taken together, these results suggest that BASCs, as defined by their surface markers, have the potential to divide in culture and are multipotent. What is needed now is evidence that these cells function as stem cells in vivo. The authors do show that dual-positive Sftpc/Scgb1a1 cells are the first cells to proliferate in the naphthalene lung injury model, which is described below. Moreover, cells with the same characteristics are expanded in adenocarcinomas of the bronchioalveolar junction that are induced by activated K-ras expression (Kim et al., 2005). However, although the identification of putative BASCs is very promising, the evidence for their self-renewal and multipotency in vivo is still preliminary.

The response of lung epithelial cells to injury

Although the normal lung turns over very slowly, it is able to respond rapidly to specific injuries that mimic damage caused by environmental or infectious agents. There is some strain dependence in the efficiency of this injury/regenerative response (e.g. Lawson et al., 2002), but each model elicits a different repair program that can reveal the proliferative and differentiation potential of the surviving cells. The experiments are frequently interpreted as showing the presence of stem cells in the adult lung. Although in some cases this may be correct, other interpretations have not always been excluded. The evidence for involvement of different cell types in the turnover and repair of the proximal and distal lung is summarized in Figs 4 and 5.

Injury by naphthalene

A popular lung injury model is the destruction of Clara cells by naphthalene. This aromatic hydrocarbon is usually administered by intraperitoneal injection, from where it reaches the lungs through the bloodstream. Only those cells that express cytochrome P4502F2 (encoded by *Cyp2f2*) are killed by the naphthalene, which is converted to toxic epoxides in such cells. Although there are some strain and dose variations in the response (Lawson et al., 2002; Plopper et al., 1992), the sequence of events is approximately as follows. Within a few hours nearly all the Clara cells die, leaving intact the few Clara cells that do not express *Cyp2f2* and are therefore resistant (Fig. 6). The ciliated cells quickly spread out, or squamate, under the dying Clara cells in an attempt to cover the basal lamina and maintain the permeability barrier of the epithelium. In doing so, they internalize and disassemble their cilia in a very specific way (Lawson et al., 2002; Van Winkle et al., 1999). Cell proliferation begins 2-3 days after injury, and by 2-4 weeks, the epithelium has returned to steady state (Stripp et al., 1995; Van Winkle et al., 1995). The NE cells appear to increase in

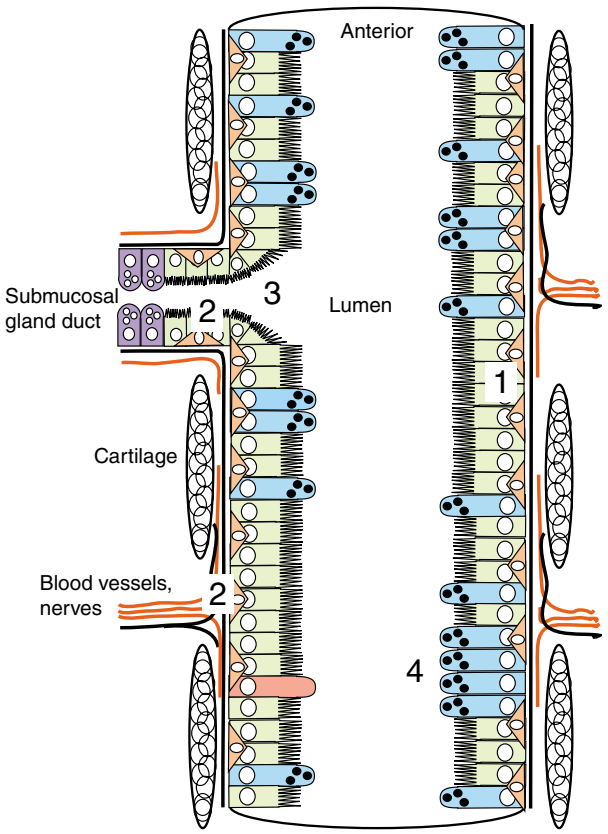

Fig. 4. Potential mechanisms involved in epithelial repair in the trachea and main bronchi. Clara-like cells are shown in blue, ciliated cells in green, neuroendocrine cells in pink, basal cells in orange and mucous cells in purple. Regions rich in blood vessels and nerves are indicated. Black line represents the basal lamina. Smooth muscle cells and fibroblasts are not shown. Putative regenerative cell populations are numbered 1-4. (**1**) K14-positive cells: these are possibly basal cells that self-renew and give rise to Clara cells and ciliated cells after destruction of Clara cells by naphthalene. (**2**) Label-retaining cells, which have been identified in submucosal gland ducts and in intercartilage regions. (**3**) Epithelial cells in submucosal glands, which can regenerate the entire luminal epithelium in grafts. (**4**) Clara cells, which can replace ciliated cells damaged by oxidants.

number after the injury through self-renewal (Reynolds et al., 2000a; Reynolds et al., 2000b; Stevens et al., 1997), a process that may be driven by sonic hedgehog (Shh) signaling (Watkins et al., 2003). However, different mechanisms appear to operate for the renewal of the Clara cells and increase in ciliated cells, depending on the region of the lung in which the repair occurs, as discussed below.

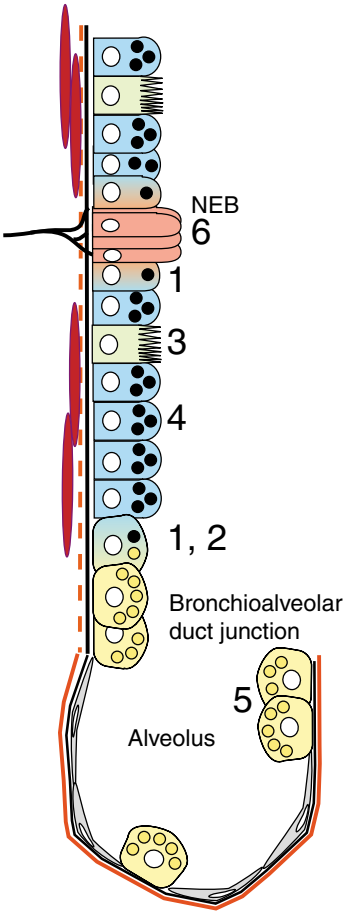

Fig. 5. Potential repair mechanisms in the distal bronchi, bronchioles and alveoli. Type II alveolar cells are in yellow, type I alveolar cells are in gray, smooth muscle cells are in red. The broken red line indicates that blood vessels are associated with the bronchiolar epithelium but not obviously enriched in particular regions. In the alveoli, blood vessels are tightly apposed to type I cells. Putative regenerative cell populations are as follows (1-6). (**1**) Variant Clara (Clara^{V}) cells adjacent to neuroendocrine bodies (NEBs) and at bronchioalveolar junction (blue/pink or blue/yellow), which are resistant to naphthalene and proliferate soon after injury by this agent. If all Clara cells are destroyed, there is no repair and the mice die. (**2**) Putative bronchioalveolar stem cells (BASCs) (blue/yellow), which self-renew in culture after flow sorting and give rise to multiple lineages in culture. These may be the same as Clara^{V} cells. (**3**) Ciliated cells, which can proliferate and transdifferentiate into Clara cells after naphthalene injury but other evidence argues against this. (**4**) Clara cells, which proliferate after NO_2 injury and give rise to ciliated cells but it is not known whether all Clara cells have this potential. (**5**) Type II cells, which give rise to type I cells after bleomycin injury to type I cells. (**6**) Neuroendocrine cells, where present, can self-renew and proliferate but do not give rise to other lineages.

Response to naphthalene injury in the proximal lung

In the trachea and main bronchi, there is good evidence that basal cells function as stem cells in repairing the destruction of the Clara cells by naphthalene. This evidence comes from the first in vivo genetic lineage labeling studies to be carried out in the adult lung, illustrating the power of this approach (Hong et al., 2004a; Hong et al., 2004b). A tamoxifen-inducible Cre recombinase under the control of a cytokeratin 14 (*K14*; *Krt1-14* – Mouse Genome Informatics) promoter was activated in mice after the naphthalene injury to drive genomic rearrangement of the *Rosa26R* reporter allele. This results in constitutive *lacZ* expression in a subset of the *K14*-expressing cells, and all of their descendents. At the end of the recovery period, the repaired epithelium contained patches of β-galactosidase (*lacZ*)-positive epithelial cells. These patches contained three different cell types: K14-positive basal cells, Clara-like cells and ciliated cells. This suggests that K-14-positive cells can self-renew and produce cells of other lineages in response to injury; in other words, they can behave like dedicated stem cells. It is tempting to conclude that the K14-positive, proliferating cells are basal cells, because under steady-state conditions *K14* is expressed exclusively by this population. However, other studies have shown that proliferating cells in repairing tracheal epithelium can activate *K14* de novo, even when they are not derived from basal cells (Liu et al., 1994). As the tamoxifen was given after the injury, these experiments strongly support the idea that basal cells are stem cells but they do not formally rule out a role for other cells in which K14 expression is activated during the injury response. In addition, they do not exclude a role for columnar cells (including ciliated cells) in the repair process.

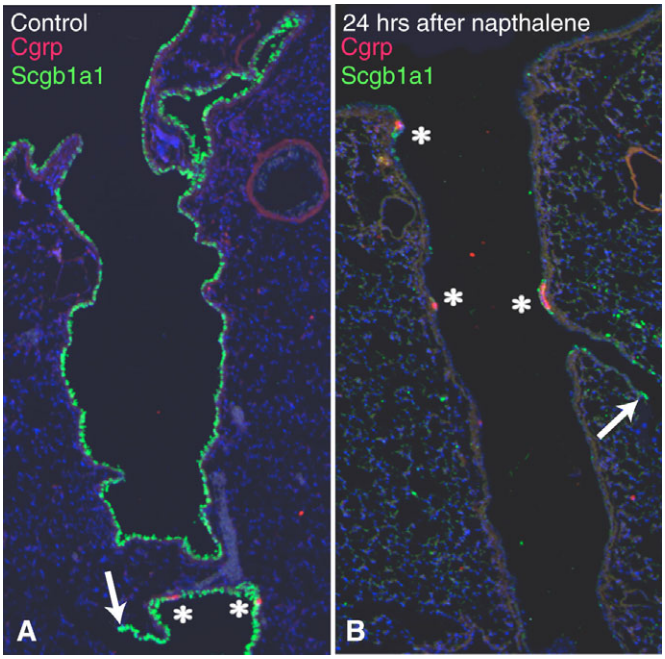

Fig. 6. Histological changes in the distal airway exposed to naphthalene. Changes in the mouse lung after exposure to naphthalene. (**A**) Section of a distal adult airway from a naphthalene-exposed mouse, showing abundant Clara cells (detected with antibody to Scgb1a1) and clusters of neuroendocrine (NE) cells (asterisk, stained with antibody to Cgrp). (**B**) A similar airway 24 hours after naphthalene exposure. Most Clara cells are lost, except for resistant cells close to NE cells and at the bronchioalveolar junctions (marked in both panels by white arrows). Images kindly provided by Adam Giangreco, Susan Reynolds and Barry Stripp (University of Pittsburgh, PA, USA).

Response to naphthalene injury in the distal lung

In the more distal lung (Figs 2, 5), where there are no basal cells, there is evidence that the Clara cell population is restored by the proliferation and self-renewal of a small number of resistant or 'variant' (Clara^{V}) cells that survive naphthalene injury. These are a label-retaining subpopulation of Clara cells located adjacent to clusters of neuroendocrine bodies (NEBs) in the bronchi/bronchioles (Hong et al., 2001; Stripp et al., 1995) or at the BADJs, where there are very few NEBs (Giangreco et al., 2002; Hong et al., 2001; Stripp et al., 1995). The latter may be the same as the putative BASCs discussed earlier that co-express Sftpc and Scgb1a1, and proliferate after naphthalene injury (Kim et al., 2005) (Figs 2, 5). Clara^{V} cells are resistant to naphthalene injury because they do not express the enzyme Cyp2f2, a member of the cytochrome P450 family that converts naphthalene to a toxic product. This lack of expression may reflect a less differentiated state of the cells relative to the majority of the Clara cell population. If Clara^{V} (and putative BASCs) can be shown to self-renew and give rise to differentiated progeny during the slow turnover of the normal lung, then they could be classified as dedicated stem cells, rather than as facultative stem cells that are called into action only in response to injury (see Box 1). They would differ from more 'classical' undifferentiated stem cells (Fig. 1) only in that they express markers of differentiated cell types (calcitonin gene-related peptide and Scgb1a1 in the case of Clara^{V} cells, and Sftpc and Scgb1a1 in the case of BASCs). The potential role of Clara^{V} cells in homeostasis and repair of the distal lung is illustrated in Fig. 7.

The precise mechanisms that underlie the self-renewal of Clara^{V} cells and the lineage diversification of their progeny are currently unknown. In addition, the question remains as to whether Clara^{V} (and BASCs) are the only cells that self renew in the steady state and after naphthalene injury. To address this issue, Reynolds and colleagues killed all of the Clara cells in the lung by giving ganciclovar to a transgenic mouse strain expressing the herpes simplex thymidine kinase specifically in Clara cells (Hong et al., 2001; Reynolds et al., 2000b). After this procedure, the airways could not be repaired. This result argues that ciliated cells cannot give rise to Clara cells, but it does not exclude the self-renewal of ciliated cells. Indeed, the role of ciliated cells in the response to naphthalene injury is contentious. In one set of studies, no evidence for proliferation was seen (Reynolds et al., 2000a). However, others have claimed that the squamated (flattened) ciliated cells do indeed proliferate 48 hours after naphthalene injury (Park et al., 2005). Moreover, they argue that some of these cells undergo transdifferentiation to non-ciliated columnar cells. In other words, the authors of this study claim that, after injury, the ciliated cells can both self-renew and give rise to another lineage, i.e. they behave as stem cells. If ciliated cells do not normally self-renew and are quiescent, they could be considered, under these conditions, to be facultative stem cells. The only way to determine for certain whether ciliated cells do indeed transdifferentiate after naphthalene injury, and whether Clara^{V} and BASCs are the only cells that can function as stem cells in the steady state and after injury is to carry out lineage tracing studies in vivo.

Repair after damage by inhaled oxidants

Although naphthalene injury has been used to study the regeneration of Clara cells in the lung, other models specifically destroy ciliated cells or type I alveolar cells. These include inhalation of oxidants such as NO_2 and ozone (which selectively kill ciliated cells) and administration of the chemotherapy agent, bleomycin (which kills type I cells). To summarize briefly (see Figs 4 and 5), these studies suggest that ciliated cells can be regenerated from Clara cells, and type II cells give rise to type I cells. Again, these findings need to be confirmed by in vivo lineage studies (Aso et al., 1976; Barth and Muller, 1999; Evans et al., 1975; Evans et al., 1986). It is also unclear whether all type II cells have the capacity to give rise to type I cells, or only those in specific regions, such as BASCs.

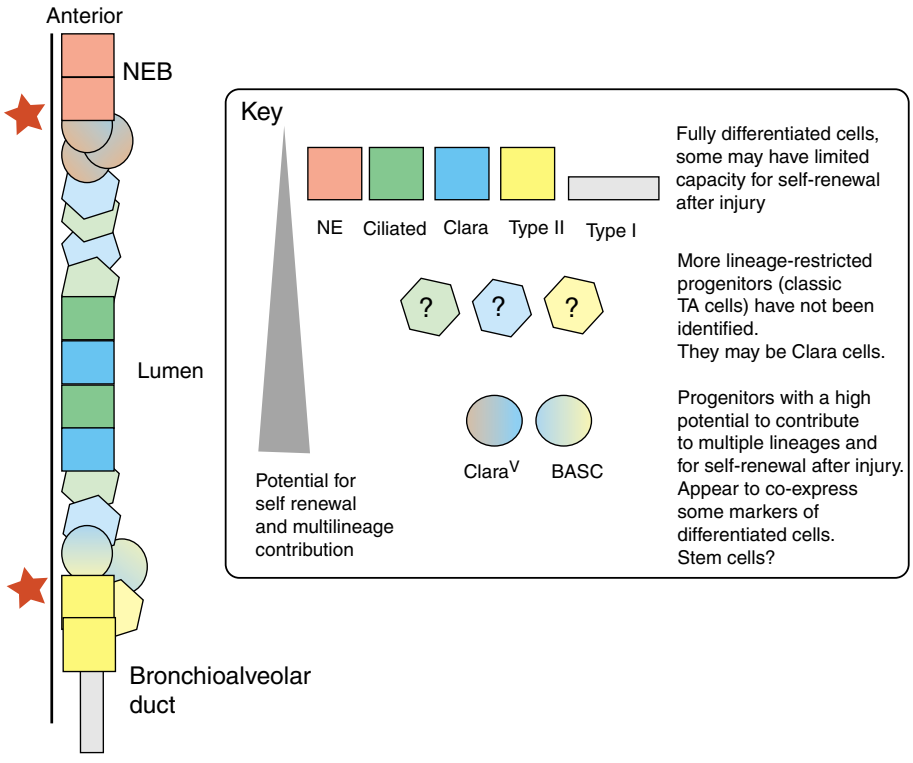

Fig. 7. A model showing putative stem cells in the distal lung. Left: depiction of the epithelial cell layer, with cells resting on a basal lamina (black line) and facing the lumen to the right. Stem cells are located in specific regions (red stars): Clara^{V} cells near neuroendocrine bodies (NEBs) and putative bronchioalveolar stem cells (BASCs) at the bronchioalveolar duct junction. Key indicates the different cell types possibly involved in steady-state turnover and injury response. The Clara^{V} and putative BASCs (which may be the same cells or members of the same stem cell population) differ from classical stem cells in that they co-express proteins also expressed by fully differentiated cell types. The existence of putative transit amplifying (TA) cells (hexagons), intermediate between stem cells and fully differentiated cells (squares) is hypothetical. Alternatively, Clara cells may be the TA population. A gradation of potential for self-renewal and contribution to different lineages might exist throughout the epithelium, with Clara^{V} and BASCs having the highest potential and fully differentiated cells the lowest.

Injury by sulfur dioxide

Both the naphthalene and oxidant exposure models injure only subsets of epithelial cells in the lung. To produce more extensive damage, investigators have used SO_2 inhalation in mice (Borthwick et al., 2001). This destroys the majority of the pseudostratified epithelial cells in the upper trachea, leaving behind protected cells in the surface layer and submucosal glands, and in patches of denuded basement membrane. Within 7 days, full repair has taken place and a morphologically normal epithelium is re-established. To identify any dedicated stem cells in this model, the investigators relied on the assumption that such cells divide infrequently and retain a DNA label over a long chase period. They therefore exposed experimental animals to repeated rounds of SO_2 and BrdU, so that almost every epithelial cell became labeled. After a chase of up to 95 days (~3.5 months), small groups of label retaining cells (LRCs) were localized either to the collecting ducts of the submucosal glands or to the surface epithelium in the inter-cartilage regions. Morphologically, the LRCs in both regions appear to be basal cells. Evidence that these cells actually proliferate in response to injury, self renew and give rise to ciliated and Clara cells in vivo awaits lineage-tracing studies. However, the idea is supported by the cell culture and naphthalene recovery experiments discussed earlier. Moreover, a xenograft model in which the surface epithelium was completely denuded, leaving behind the submucosal glands, showed that cells in the ducts of these glands can repopulate the entire tracheal surface (Borthwick et al., 2001).

Potential niches for lung stem cells

In the experiments described above, LRCs appear to cluster in the intercartilage regions, which are particularly well supplied with blood vessels and nerves. Indeed, it has been suggested that these non-epithelial cells are components of a special 'tracheal niche' that regulates the activity of dedicated stem cells (Borthwick et al., 2001). More distally, it has been suggested that the NEBs may serve as a niche for the Clara^V cells, and that the BADJ region may serve as a niche for the putative BASCs (Giangreco et al., 2002; Reynolds et al., 2000a). Both of these regions are well supplied with blood vessels, and the NEBs are innervated. These ideas are certainly in line with studies in other systems such as the hair follicle, intestine, bone marrow and brain, where there is good molecular evidence for regulatory signaling between the stem cells and surrounding (non-epithelial) cells (Botchkarev and Sharov, 2004; Calvi et al., 2003; He et al., 2004; Lie et al., 2005). For example, many of the genes active in hair follicle stem cells are components of the Wnt, Bmp and Fgf intercellular signaling pathways (Rendl et al., 2005; Tumbar et al., 2004). These have been shown to play crucial roles in intercellular signaling in lung development (Bellusci et al., 1997; Cardoso and Lu, 2006; Del Moral et al., 2006; Eblaghie et al., 2006; Shu et al., 2005; Weaver et al., 2003; Weaver et al., 2000) and are likely components of any localized stem cell niche in the trachea and distal airways. However, it should also be noted that blood vessels and nerves are closely associated with the basal lamina that underlies the epithelium along the entire airway system. Consequently, signaling between these two populations and also inflammatory cells could also regulate the potential self-renewal of differentiated or TA cells during the response to injuries and inflammation.

It is in this area of research – the control of stem cell behavior by the niche – that developmental biology is likely to have a particularly strong impact. For example, there is mounting evidence for the importance of endothelial cell signaling to adult stem cells (Paris et al., 2001; Shen et al., 2004). Developmentally, there are now clear examples of endothelial cells that regulate the proliferation and differentiation of organ primordia (liver, pancreas) and also organ morphogenesis (kidney) (Jacquemin et al., 2006; Lammert et al., 2003; Matsumoto et al., 2001; Yoshitomi and Zaret, 2004). Similarly, developmental biologists have elucidated complex gene regulatory networks involving crosstalk between a variety of signaling pathways that control progenitor cell proliferation and differentiation (Levine and Davidson, 2005). It is clear that highly related networks of interacting genes and signaling pathways regulate stem cell self-renewal versus differentiation and quiescence versus proliferation (Ivanova et al., 2002; Ramalho-Santos et al., 2002); studies in one system can only promote understanding in the other.

Role of other cell lineages in epithelial repair?

Several studies have suggested that cells from the bone marrow can differentiate into lung epithelial cells after intravenous injection into a mouse that has received a lung injury (Kotton et al., 2001; Krause et al., 2001; Macpherson et al., 2005; Theise et al., 2002). Indeed, it has been claimed that these exogenous cells can transdifferentiate into alveolar type II and I cells. Naturally, these findings have given rise to the hope that human lung diseases will one day be treated using stem cells derived from the bone marrow. However, these results in the lung, as in other tissues, are still very controversial (Raff, 2004). The role of exogenous cells in lung repair was recently tested using donor bone marrow-derived cells from a mouse line carrying in its genome a transgene that drives GFP from a type II alveolar cell-specific (*Sftpc*) promoter. Sophisticated imaging techniques demonstrated that the bone marrow-derived cells did indeed engraft in the lung at a low frequency. However, these cells did not express the lung-specific transgene (Chang et al., 2005). These data argue against the donor cells contributing directly to Sftpc-expressing host lung epithelium.

In spite of their apparent inability to contribute to functional lung epithelium directly, it is still possible that bone marrow-derived cells can aid in the repair of chemically induced lung injury (Ortiz et al., 2003; Rojas et al., 2005). For example, myelosupressed mice-treated with bleomycin and subsequently with an injection of bone marrow derived-cells had a better survival rate and lung morphology than controls (Rojas et al., 2005). The exact role of the bone marrow-derived cells is not yet clear, although their effect is apparently out of all proportion to the number that actually engraft into the lung. Perhaps these cells can secrete factors that affect the endogenous lung epithelial cells, raising the interesting possibility that circulating cells are part of the stem cell 'niche' discussed above. Indeed, recent work on mechanical injury in the colon has demonstrated that the macrophages (themselves activated by the natural gut microorganisms) provide an essential signal to activate proliferation of the stem cells (Pull et al., 2005). Such studies are important for the lung because, in the short term at least, it may be easier to modulate the composition of the niche to promote repair than to provide exogenous cells to replace the injured epithelium.

Conclusions

The picture that is emerging from studies on the response of the lung to injury is that the organ makes use of several different strategies for homeostasis and repair. In the proximal lung (trachea and main bronchi) it is likely that undifferentiated basal cells can function as classical stem cells, both self-renewing and giving rise to ciliated and

secretory cells. However, Clara cells can also give rise to ciliated cells after these are damaged by oxidants, and the potential self-renewal of Clara cells and even ciliated cells in the steady state has not been ruled out. In the more distal lung, where there are no basal cells, the evidence suggests that subpopulations of Clara cells (Clara^{V} and BASCs) in specific micro-environments can self-renew and give rise to different cell types after injury. Whether they behave as dedicated stem cells in the steady state is not yet known, and whether ciliated cells can proliferate and transdifferentiate, are still unanswered questions (Fig. 7). Finally, in the alveoli, damaged type I cells can be restored from type II cells, although whether all type II cells have this capacity is not yet known. The diversity of strategies for repair, and the different classes of stem cells in the lung, are in sharp contrast to the situation in the intestine. Here, only a few stem cells are present near the base of the crypts, and they and the TA population derived from them appear to be responsible for replenishing the entire epithelium. This difference probably reflects the fact that cell turnover in the lung is normally very low, so that a conveyor belt type mechanism for constantly and rapidly renewing the epithelium is not required. However, when injury to the epithelium of the lung does occur it has to be repaired as quickly as possible. In this case, a situation in which multiple cell types can proliferate and function as progenitors for repair is probably an advantage.

Although a general picture of epithelial turnover and repair in the lung is gradually emerging, many challenges still remain. For example, we have indicated in several sections the need for more phenotypic markers for lung cells to allow their unambiguous identification and efficient sorting by flow cytometry. There may still be important subpopulations of epithelial cells that are completely missed. We also need more genetic tools to follow cell fate and lineage relationships both in the embryo and the adult. Another pressing challenge is devising better systems to assay the developmental potential of isolated adult lung epithelial cells and rapidly testing the function of genes activated or repressed in the process of repair. Finally, we need to know more about the interactions between epithelial and non-epithelial cell populations during homeostasis and in response to injury. Answers to these and other questions are likely to come from many different directions. Among these are the application of ideas and principles derived from basic research in developmental biology.

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References

Alison, M. R., Vig, P., Russo, F., Bigger, B. W., Amofah, E., Themis, M. and Forbes, S. (2004). Hepatic stem cells: from inside and outside the liver? *Cell Prolif.* **37**, 1-21.

Aso, Y., Yoneda, K. and Kikkawa, Y. (1976). Morphologic and biochemical study of pulmonary changes induced by bleomycin in mice. *Lab. Invest.* **35**, 558-568.

Avril-Delplanque, A., Casal, I., Castillon, N., Hinnrasky, J., Puchelle, E. and Peault, B. (2005). Aquaporin-3 expression in human fetal airway epithelial progenitor cells. *Stem Cells* **23**, 992-1001.

Barth, P. J. and Muller, B. (1999). Effects of nitrogen dioxide exposure on Clara cell proliferation and morphology. *Pathol. Res. Pract.* **195**, 487-493.

Bellusci, S., Grindley, J., Emoto, H., Itoh, N. and Hogan, B. L. (1997). Fibroblast growth factor 10 (FGF10) and branching morphogenesis in the embryonic mouse lung. *Development* **124**, 4867-4878.

Blenkinsopp, W. K. (1967). Proliferation of respiratory tract epithelium in the rat. *Exp. Cell Res.* **46**, 144-154.

Borthwick, D. W., Shahbazian, M., Krantz, Q. T., Dorin, J. R. and Randell, S. H. (2001). Evidence for stem-cell niches in the tracheal epithelium. *Am. J. Respir. Cell Mol. Biol.* **24**, 662-670.

Botchkarev, V. A. and Sharov, A. A. (2004). BMP signaling in the control of skin development and hair follicle growth. *Differentiation* **72**, 512-526.

Breuer, R., Zajicek, G., Christensen, T. G., Lucey, E. C. and Snider, G. L. (1990). Cell kinetics of normal adult hamster bronchial epithelium in the steady state. *Am. J. Respir. Cell Mol. Biol.* **2**, 51-58.

Calvi, L. M., Adams, G. B., Weibrecht, K. W., Weber, J. M., Olson, D. P., Knight, M. C., Martin, R. P., Schipani, E., Divieti, P., Bringhurst, F. R. et al. (2003). Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* **425**, 841-846.

Cardoso, W. V. and Lu, J. (2006). Regulation of early lung development: questions, facts and controversies. *Development* **133**, 1611-1624.

Chang, J. C., Summer, R., Sun, X., Fitzsimmons, K. and Fine, A. (2005). Evidence that bone marrow cells do not contribute to the alveolar epithelium. *Am. J. Respir. Cell Mol. Biol.* **33**, 335-342.

Daniely, Y., Liao, G., Dixon, D., Linnoila, R. I., Lori, A., Randell, S. H., Oren, M. and Jetten, A. M. (2004). Critical role of p63 in the development of a normal esophageal and tracheobronchial epithelium. *Am. J. Physiol. Cell Physiol.* **287**, C171-C181.

Del Moral, P. M., Sala, F. G., Tefft, D., Shi, W., Keshet, E., Bellusci, S. and Warburton, D. (2006). VEGF-A signaling through Flk-1 is a critical facilitator of early embryonic lung epithelial to endothelial crosstalk and branching morphogenesis. *Dev. Biol.* **290**, 177-188.

Donnelly, G. M., Haack, D. G. and Heird, C. S. (1982). Tracheal epithelium: cell kinetics and differentiation in normal rat tissue. *Cell Tissue Kinet.* **15**, 119-130.

Dor, Y., Brown, J., Martinez, O. I. and Melton, D. A. (2004). Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* **429**, 41-46.

Dupuit, F., Gaillard, D., Hinnrasky, J., Mongodin, E., de Bentzmann, S., Copreni, E. and Puchelle, E. (2000). Differentiated and functional human airway epithelium regeneration in tracheal xenografts. *Am. J. Physiol. Lung Cell Mol. Physiol.* **278**, L165-L176.

Eblaghie, M. C., Reedy, M., Oliver, T., Mishina, Y. and Hogan, B. L. (2006). Evidence that autocrine signaling through Bmpr1a regulates the proliferation, survival and morphogenetic behavior of distal lung epithelial cells. *Dev. Biol.* **291**, 67-82.

Engelhardt, J. F., Schlossberg, H., Yankaskas, J. R. and Dudus, L. (1995). Progenitor cells of the adult human airway involved in submucosal gland development. *Development* **121**, 2031-2046.

Evans, C. M., Williams, O. W., Tuvim, M. J., Nigam, R., Mixides, G. P., Blackburn, M. R., DeMayo, F. J., Burns, A. R., Smith, C., Reynolds, S. D. et al. (2004). Mucin is produced by clara cells in the proximal airways of antigen-challenged mice. *Am. J. Respir. Cell Mol. Biol.* **31**, 382-394.

Evans, M. J., Cabral, L. J., Stephens, R. J. and Freeman, G. (1975). Transformation of alveolar type 2 cells to type 1 cells following exposure to NO2. *Exp. Mol. Pathol.* **22**, 142-150.

Evans, M. J., Shami, S. G., Cabral-Anderson, L. J. and Dekker, N. P. (1986). Role of nonciliated cells in renewal of the bronchial epithelium of rats exposed to NO2. *Am. J. Pathol.* **123**, 126-133.

Ford, J. R. and Terzaghi-Howe, M. (1992). Characteristics of magnetically separated rat tracheal epithelial cell populations. *Am. J. Physiol.* **263**, L568-L574.

Fuchs, E., Tumbar, T. and Guasch, G. (2004). Socializing with the neighbors: stem cells and their niche. *Cell* **116**, 769-778.

Giangreco, A., Reynolds, S. D. and Stripp, B. R. (2002). Terminal bronchioles harbor a unique airway stem cell population that localizes to the bronchoalveolar duct junction. *Am. J. Pathol.* **161**, 173-182.

Hayashi, T., Ishii, A., Nakai, S. and Hasegawa, K. (2004). Ultrastructure of goblet-cell metaplasia from Clara cell in the allergic asthmatic airway inflammation in a mouse model of asthma in vivo. *Virchows Arch.* **444**, 66-73.

He, X. C., Zhang, J., Tong, W. G., Tawfik, O., Ross, J., Scoville, D. H., Tian, Q., Zeng, X., He, X., Wiedemann, L. M. et al. (2004). BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt-beta-catenin signaling. *Nat. Genet.* **36**, 1117-1121.

Hong, K. U., Reynolds, S. D., Giangreco, A., Hurley, C. M. and Stripp, B. R. (2001). Clara cell secretory protein-expressing cells of the airway neuroepithelial body microenvironment include a label-retaining subset and are critical for epithelial renewal after progenitor cell depletion. *Am. J. Respir. Cell Mol. Biol.* **24**, 671-681.

Hong, K. U., Reynolds, S. D., Watkins, S., Fuchs, E. and Stripp, B. R. (2004a). Basal cells are a multipotent progenitor capable of renewing the bronchial epithelium. *Am. J. Pathol.* **164**, 577-588.

Hong, K. U., Reynolds, S. D., Watkins, S., Fuchs, E. and Stripp, B. R. (2004b). In vivo differentiation potential of tracheal basal cells: evidence for multipotent and unipotent subpopulations. *Am. J. Physiol. Lung Cell Mol. Physiol.* **286**, L643-L649.

Ivanova, N. B., Dimos, J. T., Schaniel, C., Hackney, J. A., Moore, K. A. and Lemischka, I. R. (2002). A stem cell molecular signature. *Science* **298**, 601-604.

Jacquemin, P., Yoshitomi, H., Kashima, Y., Rousseau, G. G., Lemaigre, F. P. and Zaret, K. S. (2006). An endothelial-mesenchymal relay pathway regulates early phases of pancreas development. *Dev. Biol.* **290**, 189-199.

Johnson, N. F. and Hubbs, A. F. (1990). Epithelial progenitor cells in the rat trachea. *Am. J. Respir. Cell Mol. Biol.* **3**, 579-585.

Kai, T. and Spradling, A. (2004). Differentiating germ cells can revert into functional stem cells in Drosophila melanogaster ovaries. *Nature* **428**, 564-569.

Kauffman, S. L. (1980). Cell proliferation in the mammalian lung. *Int. Rev. Exp. Pathol.* **22**, 131-191.
Keller, R. (2005). Cell migration during gastrulation. *Curr. Opin. Cell Biol.* **17**, 533-541.
Khoor, A., Stahlman, M. T., Gray, M. E. and Whitsett, J. A. (1994). Temporal-spatial distribution of SP-B and SP-C proteins and mRNAs in developing respiratory epithelium of human lung. *J. Histochem. Cytochem.* **42**, 1187-1199.
Kim, C. F., Jackson, E. L., Woolfenden, A. E., Lawrence, S., Babar, I., Vogel, S., Crowley, D., Bronson, R. T. and Jacks, T. (2005). Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* **121**, 823-835.
Kotton, D. N., Ma, B. Y., Cardoso, W. V., Sanderson, E. A., Summer, R. S., Williams, M. C. and Fine, A. (2001). Bone marrow-derived cells as progenitors of lung alveolar epithelium. *Development* **128**, 5181-5188.
Krause, D. S., Theise, N. D., Collector, M. I., Henegariu, O., Hwang, S., Gardner, R., Neutzel, S. and Sharkis, S. J. (2001). Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* **105**, 369-377.
Kurita, T., Medina, R. T., Mills, A. A. and Cunha, G. R. (2004). Role of p63 and basal cells in the prostate. *Development* **131**, 4955-4964.
Lammert, E., Cleaver, O. and Melton, D. (2003). Role of endothelial cells in early pancreas and liver development. *Mech. Dev.* **120**, 59-64.
Lanza, R. (2006). *Essentials of Stem Cell Biology*. New York: Academic Press.
Lawson, G. W., Van Winkle, L. S., Toskala, E., Senior, R. M., Parks, W. C. and Plopper, C. G. (2002). Mouse strain modulates the role of the ciliated cell in acute tracheobronchial airway injury-distal airways. *Am. J. Pathol.* **160**, 315-327.
Levine, M. and Davidson, E. H. (2005). Gene regulatory networks for development. *Proc. Natl. Acad. Sci. USA* **102**, 4936-4942.
Li, J., Tzu, J., Chen, Y., Zhang, Y. P., Nguyen, N. T., Gao, J., Bradley, M., Keene, D. R., Oro, A. E., Miner, J. H. et al. (2003). Laminin-10 is crucial for hair morphogenesis. *EMBO J.* **22**, 2400-2410.
Lie, D. C., Colamarino, S. A., Song, H. J., Desire, L., Mira, H., Consiglio, A., Lein, E. S., Jessberger, S., Lansford, H., Dearie, A. R. et al. (2005). Wnt signalling regulates adult hippocampal neurogenesis. *Nature* **437**, 1370-1375.
Liu, J. Y., Nettesheim, P. and Randell, S. H. (1994). Growth and differentiation of tracheal epithelial progenitor cells. *Am. J. Physiol.* **266**, L296-L307.
Macpherson, H., Keir, P., Webb, S., Samuel, K., Boyle, S., Bickmore, W., Forrester, L. and Dorin, J. (2005). Bone marrow-derived SP cells can contribute to the respiratory tract of mice in vivo. *J. Cell Sci.* **118**, 2441-2450.
Matsumoto, K., Yoshitomi, H., Rossant, J. and Zaret, K. S. (2001). Liver organogenesis promoted by endothelial cells prior to vascular function. *Science* **294**, 559-563.
Metzger, D. and Chambon, P. (2001). Site- and time-specific gene targeting in the mouse. *Methods* **24**, 71-80.
Nguyen, N. M., Kelley, D. G., Schlueter, J. A., Meyer, M. J., Senior, R. M. and Miner, J. H. (2005). Epithelial laminin alpha5 is necessary for distal epithelial cell maturation, VEGF production, and alveolization in the developing murine lung. *Dev. Biol.* **282**, 111-125.
Ortiz, L. A., Gambelli, F., McBride, C., Gaupp, D., Baddoo, M., Kaminski, N. and Phinney, D. G. (2003). Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. *Proc. Natl. Acad. Sci. USA* **100**, 8407-8411.
Overturf, K., Al-Dhalimy, M., Finegold, M. and Grompe, M. (1999). The repopulation potential of hepatocyte populations differing in size and prior mitotic expansion. *Am. J. Pathol.* **155**, 2135-2143.
Pack, R. J., Al-Ugaily, L. H. and Morris, G. (1981). The cells of the tracheobronchial epithelium of the mouse: a quantitative light and electron microscope study. *J. Anat.* **132**, 71-84.
Paris, F., Fuks, Z., Kang, A., Capodieci, P., Juan, G., Ehleiter, D., Haimovitz-Friedman, A., Cordon-Cardo, C. and Kolesnick, R. (2001). Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. *Science* **293**, 293-297.
Park, K. S., Wells, J. M., Zorn, A. M., Wert, S. E., Laubach, V. E., Fernandez, L. G. and Whitsett, J. A. (2005). Transdifferentiation of ciliated cells during repair of the respiratory epithelium. *Am. J. Respir. Cell Mol. Biol.* **34**, 151-157.
Pearton, D. J., Yang, Y. and Dhouailly, D. (2005). Transdifferentiation of corneal epithelium into epidermis occurs by means of a multistep process triggered by dermal developmental signals. *Proc. Natl. Acad. Sci. USA* **102**, 3714-3719.
Plopper, C. G., Suverkropp, C., Morin, D., Nishio, S. and Buckpitt, A. (1992). Relationship of cytochrome P-450 activity to Clara cell cytotoxicity. I. Histopathologic comparison of the respiratory tract of mice, rats and hamsters after parenteral administration of naphthalene. *J. Pharmacol. Exp. Ther.* **261**, 353-363.
Potten, C. S. (2004). Development of epithelial stem cell concepts. In *Handbook of Stem Cells: Vol. 2 Adult and Fetal Stems Cell* (ed. R. Lanza), pp. 1-12. New York: Academic Press.
Pull, S. L., Doherty, J. M., Mills, J. C., Gordon, J. I. and Stappenbeck, T. S. (2005). Activated macrophages are an adaptive element of the colonic epithelial progenitor niche necessary for regenerative responses to injury. *Proc. Natl. Acad. Sci. USA* **102**, 99-104.
Raff, M. (2003). Adult stem cell plasticity: fact or artifact? *Annu. Rev. Cell Dev. Biol.* **19**, 1-22.
Ramalho-Santos, M., Yoon, S., Matsuzaki, Y., Mulligan, R. C. and Melton, D. A. (2002). 'Stemness': transcriptional profiling of embryonic and adult stem cells. *Science* **298**, 597-600.
Rawlins, E. L. and Hogan, B. L. (2005). Intercellular growth factor signaling and the development of mouse tracheal submucosal glands. *Dev. Dyn.* **233**, 1378-1385.
Reader, J. R., Tepper, J. S., Schelegle, E. S., Aldrich, M. C., Putney, L. F., Pfeiffer, J. W. and Hyde, D. M. (2003). Pathogenesis of mucous cell metaplasia in a murine asthma model. *Am. J. Pathol.* **162**, 2069-2078.
Rendl, M., Lewis, L. and Fuchs, E. (2005). Molecular dissection of mesenchymal-epithelial interactions in the hair follicle. *PLoS Biol.* **3**, e331.
Reynolds, S. D., Giangreco, A., Power, J. H. and Stripp, B. R. (2000a). Neuroepithelial bodies of pulmonary airways serve as a reservoir of progenitor cells capable of epithelial regeneration. *Am. J. Pathol.* **156**, 269-278.
Reynolds, S. D., Hong, K. U., Giangreco, A., Mango, G. W., Guron, C., Morimoto, Y. and Stripp, B. R. (2000b). Conditional clara cell ablation reveals a self-renewing progenitor function of pulmonary neuroendocrine cells. *Am. J. Physiol. Lung Cell Mol. Physiol.* **278**, L1256-L1263.
Reynolds, S. D., Reynolds, P. R., Pryhuber, G. S., Finder, J. D. and Stripp, B. S. (2002). Secretoglobins SCGB3A1 and SCGB3A2 define secretory cell subsets in mouse and human airways. *Am. J. Respir. Crit. Care Med.* **166**, 1498-1509.
Rojas, M., Xu, J., Woods, C. R., Mora, A. L., Spears, W., Roman, J. and Brigham, K. L. (2005). Bone marrow-derived mesenchymal stem cells in repair of the injured lung. *Am. J. Respir. Cell Mol. Biol.* **33**, 145-152.
Schoch, K. G., Lori, A., Burns, K. A., Eldred, T., Olsen, J. C. and Randell, S. H. (2004). A subset of mouse tracheal epithelial basal cells generates large colonies in vitro. *Am. J. Physiol. Lung Cell Mol. Physiol.* **286**, L631-L642.
Shackleton, M., Vaillant, F., Simpson, K. J., Stingl, J., Smyth, G. K., Asselin-Labat, M. L., Wu, L., Lindeman, G. J. and Visvader, J. E. (2006). Generation of a functional mammary gland from a single stem cell. *Nature* **439**, 84-88.
Shen, Q., Goderie, S. K., Jin, L., Karanth, N., Sun, Y., Abramova, N., Vincent, P., Pumiglia, K. and Temple, S. (2004). Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. *Science* **304**, 1338-1340.
Shu, W., Guttentag, S., Wang, Z., Andl, T., Ballard, P., Lu, M. M., Piccolo, S., Birchmeier, W., Whitsett, J. A., Millar, S. E. et al. (2005). Wnt/beta-catenin signaling acts upstream of N-myc, BMP4, and FGF signaling to regulate proximal-distal patterning in the lung. *Dev. Biol.* **283**, 226-239.
Signoretti, S., Pires, M. M., Lindauer, M., Horner, J. W., Grisanzio, C., Dhar, S., Majumder, P., McKeon, F., Kantoff, P. W., Sellers, W. R. et al. (2005). p63 regulates commitment to the prostate cell lineage. *Proc. Natl. Acad. Sci. USA* **102**, 11355-11360.
Stevens, T. P., McBride, J. T., Peake, J. L., Pinkerton, K. E. and Stripp, B. R. (1997). Cell proliferation contributes to PNEC hyperplasia after acute airway injury. *Am. J. Physiol.* **272**, L486-L493.
Stripp, B. R., Maxson, K., Mera, R. and Singh, G. (1995). Plasticity of airway cell proliferation and gene expression after acute naphthalene injury. *Am. J. Physiol.* **269**, L791-L799.
Theise, N. D., Henegariu, O., Grove, J., Jagirdar, J., Kao, P. N., Crawford, J. M., Badve, S., Saxena, R. and Krause, D. S. (2002). Radiation pneumonitis in mice: a severe injury model for pneumocyte engraftment from bone marrow. *Exp. Hematol.* **30**, 1333-1338.
Thurlbeck, W. M. (1975). Lung growth and alveolar multiplication. *Pathobiol. Annu.* **5**, 1-34.
Tumbar, T., Guasch, G., Greco, V., Blanpain, C., Lowry, W. E., Rendl, M. and Fuchs, E. (2004). Defining the epithelial stem cell niche in skin. *Science* **303**, 359-363.
Tyner, J. W., Kim, E. Y., Ide, K., Pelletier, M. R., Roswit, W. T., Morton, J. D., Battaile, J. T., Patel, A. C., Patterson, G. A., Castro, M. et al. (2006). Blocking airway mucous cell metaplasia by inhibiting EGFR antiapoptosis and IL-13 transdifferentiation signals. *J. Clin. Invest.* **116**, 309-321.
Van Winkle, L. S., Buckpitt, A. R., Nishio, S. J., Isaac, J. M. and Plopper, C. G. (1995). Cellular response in naphthalene-induced Clara cell injury and bronchiolar epithelial repair in mice. *Am. J. Physiol.* **269**, L800-L818.
Van Winkle, L. S., Johnson, Z. A., Nishio, S. J., Brown, C. D. and Plopper, C. G. (1999). Early events in naphthalene-induced acute Clara cell toxicity: comparison of membrane permeability and ultrastructure. *Am. J. Respir. Cell Mol. Biol.* **21**, 44-53.
Vu, T. H., Alemayehu, Y. and Werb, Z. (2003). New insights into saccular development and vascular formation in lung allografts under the renal capsule. *Mech. Dev.* **120**, 305-313.
Watkins, D. N., Berman, D. M., Burkholder, S. G., Wang, B., Beachy, P. A. and Baylin, S. B. (2003). Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. *Nature* **422**, 313-317.
Watt, F. M. and Hogan, B. L. M. (2000). Out of Eden: stem cells and their niches. *Science* **287**, 1427-1430.
Weaver, M., Dunn, N. R. and Hogan, B. L. (2000). Bmp4 and Fgf10 play opposing roles during lung bud morphogenesis. *Development* **127**, 2695-2704.
Weaver, M., Batts, L. and Hogan, B. L. (2003). Tissue interactions pattern the mesenchyme of the embryonic mouse lung. *Dev. Biol.* **258**, 169-184.

Wells, A. B. (1970). The kinetics of cell proliferation in the tracheobronchial epithelia of rats with and without chronic respiratory disease. *Cell Tissue Kinet.* **3**, 185-206.
Williams, O. W., Sharafkhaneh, A., Kim, V., Dickey, B. F. and Evans, C. M. (2006). Airway mucus: from production to secretion. *Am. J. Respir. Cell Mol. Biol.* **34**, 527-536.
Xin, L., Ide, H., Kim, Y., Dubey, P. and Witte, O. N. (2003). In vivo regeneration of murine prostate from dissociated cell populations of postnatal epithelia and urogenital sinus mesenchyme. *Proc. Natl. Acad. Sci. USA* **100**, 11896-11903.
Xin, L., Lawson, D. A. and Witte, O. N. (2005). The Sca-1 cell surface marker enriches for a prostate-regenerating cell subpopulation that can initiate prostate tumorigenesis. *Proc. Natl. Acad. Sci. USA* **102**, 6942-6947.
Yoshitomi, H. and Zaret, K. S. (2004). Endothelial cell interactions initiate dorsal pancreas development by selectively inducing the transcription factor Ptf1a. *Development* **131**, 807-817.